

ZIEGLER *et al.* - 09/963,521
Client/Matter: 021123-0282413

II. AMENDMENTS TO THE CLAIMS

1-18. (Cancel)

19. (New) A process for the preparation of L-threonine comprising fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* in which the *Corynebacterium glutamicum thrE* gene encoding a threonine export protein is overexpressed by increasing the copy number of said gene, and isolating said L-threonine produced by said *Corynebacterium*.

20. (New) The process of claim 19, wherein said *Corynebacterium* or *Brevibacterium* also are overexpressed by increasing the copy number of the *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase.

21. (New) The process of claim 19, wherein said *Corynebacterium* or *Brevibacterium* also are overexpressed by increasing the copy number of the *Corynebacterium glutamicum hom* gene encoding for homoserine dehydrogenase.

22. (New) The process of claim 19, wherein said *Corynebacterium* or *Brevibacterium* also are overexpressed by increasing the copy number of the *Corynebacterium glutamicum hom^{dr}* allele encoding a feedback-resistant homoserine dehydrogenase.

23. (New) The process of claim 19, wherein said *Corynebacterium* or *Brevibacterium* also are overexpressed by increasing the copy number of the *Corynebacterium glutamicum mqo* gene encoding malate:quinone oxidoreductase.

24. (New) The process of claim 19, wherein the *Corynebacterium* of the species *Corynebacterium glutamicum* are used.

ZIEGLER *et al.* - 09/963,521
Client/Matter: 021123-0282413

25. (New) The process of claim 19, wherein the *Brevibacterium* of the species *Brevibacterium flavum* are used.

26. (New) A process for the preparation of L-threonine comprising fermenting L-threonine producing coryneform bacteria in which the *Corynebacterium glutamicum* thrE gene encoding a threonine export carrier protein is overexpressed by increasing the copy number of the thrE gene, and isolating said L-threonine produced by said coryneform bacteria, wherein said coryneform bacteria have been transformed with a plasmid vector comprising the *C. glutamicum* thrE gene encoding said threonine export carrier protein and said plasmid vector is pZ1thrE, which is deposited in *Brevibacterium flavum* under deposit number DSM12840 (support page 12, line 23, 24).

27. (New) The process of claim 26, wherein said coryneform bacteria also overexpress by increasing the copy number of the *Corynebacterium glutamicum* pyc gene encoding pyruvate carboxylase.

28. (New) The process of claim 26, wherein said coryneform bacteria also overexpress by increasing the copy number of the *Corynebacterium glutamicum* hom gene encoding for homoserine dehydrogenase.

29. (New) The process of claim 26, wherein said coryneform bacteria also overexpress by increasing the copy number of the *Corynebacterium glutamicum* hom^{dr} allele encoding a feedback-resistant homoserine dehydrogenase.

30. (New) The process of claim 26, wherein said coryneform bacteria also overexpress by increasing the copy number of the *Corynebacterium glutamicum* mqo gene encoding malate:quinone oxidoreductase.

31. (New) The process of claim 26, wherein the coryneform bacteria of the genus *Corynebacterium* are used.

ZIEGLER *et al.* - 09/963,521
Client/Matter: 021123-0282413

32. (New) The process of claim 31, wherein the *Corynebacterium* of the species *Corynebacterium glutamicum* are used.

33. (New) The process of claim 26, wherein the coryneform bacteria of the genus *Brevibacterium* are used.

34. (New) The process of claim 33, wherein the *Brevibacterium* of the species *Brevibacterium flavum* are used.

35. (New) A process for the fermentative preparation of L-threonine comprising:

- (a) fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* bacteria in which a *thrE* gene encoding a threonine export carrier protein is overexpressed by increasing the copy number of said gene; and, wherein said coryneform bacteria also overexpress by increasing the copy number one or more of the coryneform genes selected from the group consisting of: the *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase, the *Corynebacterium glutamicum hom* gene encoding for homoserine dehydrogenase, the *Corynebacterium glutamicum hom^{dr}* allele encoding a feedback-resistant homoserine dehydrogenase, and the *Corynebacterium glutamicum mqo* gene encoding for malate:quinone oxidoreductase;
- (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and
- (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b).

36. (New) A process for the fermentative preparation of L-threonine comprising:

- (a) fermenting L-threonine producing coryneform bacteria in which a *thrE* gene encoding a threonine export carrier protein is overexpressed by increasing the copy number of the gene; and, wherein said coryneform bacteria also overexpress by increasing the copy number of one or

ZIEGLER *et al.* - 09/963,521
Client/Matter: 021123-0282413

more of the coryneform genes selected from the group consisting of:
the *Corynebacterium glutamicum* pyc gene encoding pyruvate
carboxylase, the *Corynebacterium glutamicum* hom gene encoding for
homoserine dehydrogenase, the *Corynebacterium glutamicum* hom^{dr}
allele encoding a feedback-resistant homoserine dehydrogenase, and
the *Corynebacterium glutamicum* mqo gene encoding for
malate:quinone oxidoreductase;

- (b) concentrating the L-threonine in the fermentation medium or in said
coryneform bacteria; and
- (c) isolating L-threonine from the fermentation medium or coryneform
bacteria of step (b)

wherein said coryneform bacteria have been transformed with a plasmid vector comprising
the *C. glutamicum* thrE gene encoding said threonine export carrier protein and said plasmid
vector is pZ1thrE, which is deposited in *Brevibacterium flavum* under deposit number
DSM12840.

37. (New) The process of claim 19, wherein said thrE gene comprises a
polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2.

38. (New) The process of claim 37, wherein said polynucleotide comprises
nucleotides 398 to 1864 of SEQ ID NO: 1.

39. (New) The process of claim 38, wherein said thrE gene comprises SEQ ID NO: 1
and SEQ ID NO: 3.

40. (New) A process for the preparation of L-threonine comprising fermenting L-
threonine producing *Corynebacterium* or *Brevibacterium* bacteria in which the
Corynebacterium glutamicum thrE gene encoding a threonine export protein is overexpressed
by operatively linking said gene to a promoter, and isolating said L-threonine produced by
said *Corynebacterium*.

41. (New) The process of claim 40, wherein said *Corynebacterium* or
Brevibacterium also are overexpressed by operatively linking the *Corynebacterium*
glutamicum pyc gene encoding pyruvate carboxylase to a promoter.

ZIEGLER *et al.* - 09/963,521
Client/Matter: 021123-0282413

42. (New) The process of claim 40, wherein said *Corynebacterium* or *Brevibacterium* also are overexpressed by operatively linking the *Corynebacterium glutamicum hom* gene encoding for homoserine dehydrogenase to a promoter.

43. (New) The process of claim 40, wherein said *Corynebacterium* or *Brevibacterium* also are overexpressed by operatively linking the *Corynebacterium glutamicum hom^{dr}* allele encoding a feedback-resistant homoserine dehydrogenase to a promoter.

44. (New) The process of claim 40, wherein said *Corynebacterium* or *Brevibacterium* also are overexpressed by operatively linking the *Corynebacterium glutamicum mqo* gene encoding malate:quinone oxidoreductase to a promoter.

45. (New) The process of claim 40, wherein the *Corynebacterium* bacteria of the species *Corynebacterium glutamicum* are used.

46. (New) The process of claim 40, wherein the *Brevibacterium* bacteria of the species *Brevibacterium flavum* are used.

47. (New) A process for the preparation of L-threonine comprising fermenting L-threonine producing coryneform bacteria in which the *Corynebacterium glutamicum thrE* gene encoding a threonine export carrier protein is overexpressed by operatively linking said *thrE* gene to a promoter, and isolating said L-threonine produced by said coryneform bacteria, wherein said coryneform bacteria have been transformed with a plasmid vector comprising the *C. glutamicum thrE* gene encoding said threonine export carrier protein and said plasmid vector is pZ1*thrE*, which is deposited in *Brevibacterium flavum* under deposit number DSM12840 (support page 12, line 23, 24).

ZIEGLER *et al.* - 09/963,521
Client/Matter: 021123-0282413

48. (New) The process of claim 47, wherein said coryneform bacteria also overexpress by operatively linking the *Corynebacterium glutamicum* *pyc* gene encoding pyruvate carboxylase to a promoter.

49. (New) The process of claim 47, wherein said coryneform bacteria also overexpress by operatively linking the *Corynebacterium glutamicum* *hom* gene encoding for homoserine dehydrogenase to a promoter.

50. (New) The process of claim 47, wherein said coryneform bacteria also overexpress by operatively linking the *Corynebacterium glutamicum* *hom^{dr}* allele encoding a feedback-resistant homoserine dehydrogenase to a promoter.

51. (New) The process of claim 47, wherein said coryneform bacteria also overexpress by operatively linking the *Corynebacterium glutamicum* *mgo* gene encoding malate:quinone oxidoreductase to a promoter.

52. (New) The process of claim 47, wherein the coryneform bacteria of the genus *Corynebacterium* are used.

53. (New) The process of claim 52, wherein the *Corynebacterium* of the species *Corynebacterium glutamicum* are used.

54. (New) The process of claim 47, wherein the coryneform bacteria of the genus *Brevibacterium* are used.

55. (New) The process of claim 54, wherein the *Brevibacterium* of the species *Brevibacterium flavum* are used.

56. (New) A process for the fermentative preparation of L-threonine comprising:

ZIEGLER *et al.* - 09/963,521
Client/Matter: 021123-0282413

- (a) fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* bacteria in which a *thrE* gene encoding a threonine export carrier protein is overexpressed by operatively linking said gene to a promoter; and, wherein said coryneform bacteria also overexpress by operatively linking one or more of the coryneform genes selected from the group consisting of: the *Corynebacterium glutamicum* *pyc* gene encoding pyruvate carboxylase, the *Corynebacterium glutamicum* *hom* gene encoding for homoserine dehydrogenase, the *Corynebacterium glutamicum* *hom^{dr}* allele encoding a feedback-resistant homoserine dehydrogenase, and the *Corynebacterium glutamicum* *mgo* gene encoding for malate:quinone oxidoreductase to a promoter;
- (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and
- (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b).

57. (New) A process for the fermentative preparation of L-threonine comprising:

- (a) fermenting L-threonine producing coryneform bacteria in which a *thrE* gene encoding a threonine export carrier protein is overexpressed by operatively linking said gene to a promoter; and, wherein said coryneform bacteria also overexpress by operatively linking one or more of the coryneform genes selected from the group consisting of: the *Corynebacterium glutamicum* *pyc* gene encoding pyruvate carboxylase, the *Corynebacterium glutamicum* *hom* gene encoding for homoserine dehydrogenase, the *Corynebacterium glutamicum* *hom^{dr}* allele encoding a feedback-resistant homoserine dehydrogenase, and the *Corynebacterium glutamicum* *mgo* gene encoding for malate:quinone oxidoreductase to a promoter;
- (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and
- (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b)

ZIEGLER *et al.* - 09/963,521
Client/Matter: 021123-0282413

wherein said coryneform bacteria have been transformed with a plasmid vector comprising the *C. glutamicum* thrE gene encoding said threonine export carrier protein and said plasmid vector is pZ1thrE, which is deposited in Brevibacterium flavum under deposit number DSM12840.

58. (New) The process of claim 40, wherein said thrE gene comprises a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2.

59. (New) The process of claim 58, wherein said polynucleotide comprises nucleotides 398 to 1864 of SEQ ID NO: 1.

60. (New) The process of claim 59, wherein said thrE gene comprises SEQ ID NO: 1 and SEQ ID NO: 3.